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Evaluation of Innate Immunity Biomarkers on Admission and at Discharge From an Acute Heart Failure Episode

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Background: The involvement of the immune system in heart failure (HF) has been demonstrated. Evidence shows that innate immunity can have a role in the remodeling process and progression of HF. With previous studies showing the prognostic value of some innate immunity markers and their relevance in this condition, we aim to evaluate how these markers vary on hospitalization due to an acute episode of HF and at discharge. *Methods:* About 154 patients admitted with acute HF were prospectively recruited. Patients were evaluated on admission and at discharge from the hospital. Patients with infection were separately analyzed. Innate immunity, inflammatory, and cardiac biomarkers were measured and were compared between groups and between admission and discharge and with reference values of biological variation. *Results:* Median patients' age was 78 years, and half of the patients were men. The median duration of hospitalization was 6 days. C3 and C4 protein levels significantly increased ($P < 0.001$) between admission and discharge, as well as eosinophils ($P < 0.001$) and BNP levels decreased ($P < 0.001$). Variation in all these variables was independent of infection and biological variation. *Conclusion:* Our results show that innate immunity markers such as C3 and C4 increase after treatment for acute HF, supporting the hypothesis that they can be involved in the resolution of the acute episode. J. Clin. Lab. Anal. **30**:1183–1190, 2016.

Key words: acute disease; biomarkers; heart failure; hospitalization; innate immunity

INTRODUCTION

The well-established risk of hospitalization and death and the continuous increase in incidence and prevalence of heart failure (HF) make this condition a considerable public health priority (1). Although brain natriuretic peptide (BNP) is a major biomarker in HF (2), it unlikely reflects all the processes involved in this condition. The activation of the immune system, alongside with other pathways, has been receiving a growing interest. Growing evidence indicates that patients with HF are characterized by a state of chronic and systemic low-grade inflammation, through the imbalance of its mediators (3, 4). Activation of innate immunity may have an even more important role in this condition, as both infectious and/or noninfectious events could be operating in HF (5, 6). Also, knowing that one of the hallmarks of HF is the remodeling process in the heart, innate immunity can have a predominant role in HF initiation and progression. In fact, after an event of acute cardiac injury, such as myocardial infarction (MI), activation of the innate immune system is a prerequisite for adequate healing (7). However, long-term chronic innate immune activation is detrimental and may lead

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to adverse left ventricular remodeling and aggravation of HF (8). Among all the innate immune system markers, the most well-studied are the toll-like receptors (TLRs), with several experimental studies showing that TLR2 and TLR4 may play a role in the progression of HF (7, 9, 10). The inflammatory biomarker C-reactive protein (CRP) (11) and other proinflammatory cytokines, such as tumor necrosis factor α (TNF-α) and at least three interleukins (IL-1β, 6, and 18), have also been associated with the failing myocardium (12, 13). Knowing that the activation of innate immunity is not limited to TLRs and that inflammation is known to be a downstream effector of the innate immune system, it is most likely that other innate immunity markers may be activated in HF. In previous studies of our group, we already demonstrated that lower levels of the complement protein C3, at the time of discharge, are a strong and independent predictor of unfavorable outcome in patients following an acute episode of HF (14). In this context, we aimed to evaluate the variation in this and other innate immunity markers in patients suffering from an episode of acute HF, upon hospitalization and at discharge.

MATERIALS AND METHODS

Subjects

A prospective observational study was conducted between January 2009 and December 2010. During this period, 154 patients hospitalized due to an acute episode of HF at the Internal Medicine Department of São João Hospital Center, Portugal, were recruited. Of the 154 patients, 21 had incomplete information, leaving 133 patients for the current analysis. From these patients, 55 were admitted with a precipitating factor of infection for HF and/or had an infection during hospitalization. These patients were separated in a different group. Infection was defined by the decision of the attending physician to treat the patient with antibiotics. Viral infection was not considered once its frequency in hospitalized patients is rather low. Worsening or de novo HF patients were included. Patients with acute coronary syndrome were excluded. Treatment and time of discharge were decided by the attending physician. Clinical and demographic data and other relevant information, such as medication, were collected through interview upon the collection of the blood samples or by consulting medical registries. Comorbidities were recorded for each patient. Coronary heart disease was defined as history of MI, history or electrocardiographic evidence of ischemia, or coronary angiography confirmation. Diabetes mellitus, anemia history, chronic arterial hypertension, and chronic renal dysfunction were defined as either the presence of previous diagnosis or prescription of pharmacological treatment. Hospitalization days were calculated by the difference between admission and discharge days. All patients provided written informed consent to participate in the study. The study protocol conforms to the ethical guidelines of the Declaration of Helsinki and was approved by the local ethics committee.

Blood sampling

Venous blood samples were collected from all patients on admission and at discharge day into serum separator and tripotassium ethylenediaminetetraacetic acid (EDTA)-coated tubes (Venosafe®, Terumo Corporation, Tokyo, Japan). An EDTA tube was used for obtaining a complete blood count (CBC). The blood samples for serum separation were allowed to clot and then centrifuged, while EDTA tubes for plasma separation were immediately centrifuged for 15 min at 2,000 × *g*. Serum and plasma samples were separated, aliquot, and stored at −80°C for further analysis.

Methods

An echocardiogram was performed within 72 h of admission to all eligible patients during hospitalization. Comprehensive echocardiographic assessment was performed using a multifrequency matrix probe (Vivid S6; GE Healthcare, Little Chalfont, UK). The diagnosis of HF was made based on the European Society of Cardiology guidelines (15). An acute HF episode was diagnosed: "de novo" HF on a first clinical presentation and chronic HF with clinical condition worsening, both requiring urgent therapy. Patients with left ventricular systolic dysfunction (LVSD) and with HF with preserved ejection fraction were included in the registry. Normal systolic fraction was defined as a left ventricular ejection fraction (LVEF) above 50%.

A CBC with leukocyte differential was obtained in an automated blood counter Sysmex XE-5000 (Emilio de Azevedo Campos, Senhora da Hora, Portugal). C1 inhibitor (C1i) and C3 and C4 complement proteins were measured in a Dimension Vista 1500 nephelometer (Siemens, Erlangen, Alemanha). CRP was measured in the automated clinical chemistry Olympus AU5400 analyzer (Beckman-Coulter, Brea, CA). BNP and Troponin I (TnI) were measured by a chemiluminescent immunoassay in the Architect i2000 automated analyzer (Abbott, Chicago, IL). IL-6 was measured by way of an electrochemiluminescent immunoassay using a Cobas E411 automated analyzer (Roche, Basel, Switzerland).

Statistical analysis

Continuous variables are presented as mean (standard deviation) or median (interquartile range) depending on their distribution. Normality of the variables was tested by the Kolmogorov–Smirnov test. Categorical variables are presented as counts and proportions.

For comparing data between the admission and the discharge date, a paired *t*-test was used for comparing normal distributed variables, while the Wilcoxon test was used for the nondistributed variables. For comparison between the group with infection and without infection, a one-way ANOVA test was used for normal distributed variables and a Mann–Whitney test was used for the nondistributed variables.

Mean percentage variation between admission and discharge was calculated for all variables by subtracting the value from discharge to the one of admission and divided by the discharge value. These variations were compared with biological variation published by Ricos et al. (16).

The *P* value considered for statistical significance was 0.05 for a confidence interval of 95%. Data were collected and analyzed using SPSS software (v20.0; SPSS Inc, Chicago, IL).

RESULTS

Median patients' age was 78 years, and approximately, half of them were men (48.1%). The most common comorbidity was chronic arterial hypertension, affecting 74.4% of the patients. Half of the patients were on statin regimen, and more than 68% were on a diuretic and/or renin–angiotensin–aldosterone system modulation regimen prior to admission. Patients' characteristics, comorbidities, and medication are presented in Table 1.

Table 2 presents the laboratory values and changes in the studied markers upon both evaluations, as well as the comparisons between admission and discharge. All of the complement proteins showed a significant increase, higher than the accepted biological variation ($P < 0.001$), from the initial hospitalization point up to discharge. Regarding the cellular counts, neutrophils, eosinophils, and lymphocytes had a higher variation than the accepted biological variation ($P < 0.001$). Inflammatory (CRP and IL-6) and cardiac injury (BNP and TnI) biomarkers are also presented in Table 2. From all these markers, only BNP and TnI changes were higher than the accepted biological variation. For IL-6, although there was a change between admission and discharge ($P = 0.037$), there are no available biological variation data to compare with.

TABLE 1. Patients' Demographics, Clinical Characteristics, Comorbidities, and Medication

	All patients ($n = 133$)
Clinical characteristics	
Age (years), median (IQR)	78 (69; 84)
Male sex, *n* (%)	64 (48.1)
Ischemic etiology of HF, *n* (%)	68 (51.1)
Atrial fibrillation etiology of HF (%)	14 (10.5)
Valvular etiology of HF	0 (0.0)
Preserved LVSF, *n* (%)	51 (38.3)
NYHA class at admission (IV/III), *n* (%)	100 (75.2)
Comorbidities	
Diabetes mellitus, *n* (%)	53 (39.8)
Anemia history, *n* (%)	53 (39.8)
Chronic arterial hypertension, *n* (%)	99 (74.4)
Chronic renal dysfunction, *n* (%)	23 (17.3)
Smoking habits, *n* (%)	
Current smoker/ex-smoker	13 (9.8)/34 (25.6)
Alcohol habits, *n* (%)	62 (46.6)
Medication prior to admission	
ACEi, *n* (%)	69 (51.9)
ARA, *n* (%)	22 (16.5)
Spironolactone, *n* (%)	19 (14.3)
Beta-blocker, *n* (%)	62 (46.6)
Nitrates, *n* (%)	32 (24.1)
Statin, *n* (%)	67 (50.4)
Diuretic, *n* (%)	92 (69.2)
Medication at discharge	
ACEi, *n* (%)	99 (74.4)
ARA, *n* (%)	15 (11.2)
Spironolactone, *n* (%)	38 (28.6)
Beta-blocker, *n* (%)	99 (74.4)
Nitrates, *n* (%)	34 (25.6)
Statin, *n* (%)	86 (64.7)
Diuretic, *n* (%)	123 (92.5)
Hospitalization days, median (IQR)	6 (4.11)

IQR, interquartile range; HF, heart failure; LVSF, left ventricular systolic function; NYHA, New York Heart Association; ACEi, angiotensin-converting enzyme inhibitor; ARA, angiotensin II receptor antagonist.

Table 3 presents the same markers in the subgroup of patients, which had infection as precipitating factor for HF or had an infection during hospitalization. Unlike for all patients, in this subgroup, the complement protein C4 showed no significant change ($P = 0.076$). Also, unlike in the previous subgroup, in this subgroup, leukocytes and basophils had higher changes than the accepted biological variation ($P < 0.001$).

The laboratory values for the subgroup of patients who did not had infection has a precipitating factor of HF or had no infection during hospitalization are presented in Table 4. Also in these patients, all complement proteins showed a significant increase between admission and discharge with higher variations than the biological variation ($P = 0.018$ for C1i and $P < 0.001$ for C3c and C4c). Regarding the inflammatory markers, only CRP showed a significant decrease,

TABLE 2. Laboratory Values at Admission and at Discharge in All Patients and Comparison Between Admission and Discharge

All patients (n = 133)	Admission	Discharge	Variation (%)	Biological variation (%)	P value
C1i (mg/dl), median (IQR)	**32.0 (28.2; 35.0)**	**33.7 (30.5; 37.4)**	**−5.3 (−15.9; 3.0)**	–	**< 0.001**
C3 (mg/dl), mean (SD)	**116.1 (25.1)**	**133.4 (33.4)**	**−20.6 (54.8)**	**5.2**	**< 0.001**
C4 (mg/dl), median (IQR)	**24.9 (19.5; 30.8)**	**28.7 (22.1; 37.5)**	**−14.3 (−41.9; 4.8)**	**8.9**	**< 0.001**
Leukocytes (cells/μl), median (IQR)	7915 (6158; 10165)	7220 (5680; 8440)	7.0 (−9.3; 25.5)	11.4	0.001
Neutrophils (cells/μl), median (IQR)	**5905 (4108; 7943)**	**4660 (3520; 5770)**	**19.5 (−4.6; 40.2)**	**17.1**	**< 0.001**
Monocytes (cells/μl), median (IQR)	510 (370; 700)	540 (440; 720)	−5.9 (−35.5; 18.3)	17.8	0.089
Eosinophils (cells/μl), median (IQR)	**55 (10; 150)**	**140 (80; 240)**	**−64.6 (−250; 1.6)**	**21.0**	**< 0.001**
Basophils (cells/μl), median (IQR)	20 (10; 20)	20 (20; 40)	−14.6 (−100.0; 0.0)	28.0	<0.001
Lymphocytes (cells/μl), median (IQR)	**1065 (710; 1603)**	**1430 (1080; 1950)**	**−20.1 (−72.1; 1.4)**	**10.2**	**< 0.001**
CRP (mg/l), median (IQR)	21.0 (10.2; 61.8)	13.2 (5.5; 25.2)	38.1 (−4.5; 76.1)	42.2	<0.001
IL-6 (pg/ml), median (IQR)	**17.3 (9.5; 38.1)**	**15.8 (8.3; 28.6)**	**24.3 (−57.1; 57.3)**	–	**0.037**
BNP (pg/ml), median (IQR)	**1287.1 (615.2; 2564.0)**	**599.0 (281.3; 1207.0)**	**48.2 (20.1; 72.9)**	**10.0**	**< 0.001**
TnI (ng/ml), median (IQR)	**0.045 (0.190; 0.128)**	**0.027 (0.014; 0.051)**	**37.8 (−0.8; 77.1)**	**14.1**	**< 0.001**
Hemoglobin (g/dl), mean (SD)	12.1 (2.1)	12.2 (2.0)	−1.5 (9.2)	2.9	0.297
Hematocrit (%), mean (SD)	37.2 (6.2)	37.4 (5.7)	−1.1 (9.4)	2.7	0.631
MCHC (g/dl), mean (SD)	32.6 (1.3)	32.7 (1.4)	−0.5 (3.2)	1.1	0.104

IQR, interquartile range; SD, standard deviation; C1i, C1 inhibitor; CRP, C-reactive protein; IL, interleukin; BNP, B-type natriuretic peptide; TnI, Troponin I; MCHC, mean corpuscular hemoglobin concentration.
Variation represents the median difference value between the admission and discharge values.

TABLE 3. Laboratory Values at Admission and at Discharge in Patients with Infection and Comparison Between Admission and Discharge

Infected (n = 55)	Admission	Discharge	Variation (%)	Biological variation (%)	P value
C1i (mg/dl), median (IQR)	**32.9 (29.6; 36.2)**	**36.3 (31.8; 39.6)**	**−6.7 (−26.1; 0.9)**	–	**< 0.001**
C3 (mg/dl), mean (SD)	**119.0 (26.3)**	**137.0 (31.0)**	**−31.7 (77.4)**	**5.2**	**< 0.001**
C4 (mg/dl), median (IQR)	26.5 (19.6; 31.3)	28.9 (20.8; 38.9)	−7.8 (−39.8; 14.7)	8.9	0.076
Leukocytes (cells/μl), median (IQR)	**8845 (6080; 11638)**	**7436 (5918; 9263)**	**14.1 (−8.4; 30.8)**	**11.4**	**0.002**
Neutrophils (cells/μl), median (IQR)	**7170 (4858; 9125)**	**5250 (3508; 6505)**	**26.1 (−0.1; 44.2)**	**17.1**	**< 0.001**
Monocytes (cells/μl), median (IQR)	520 (370; 763)	555 (460; 783)	−5.9 (−27.6; 21.3)	17.8	0.743
Eosinophils (cells/μl), median (IQR)	**20 (0; 138)**	**145 (88; 263)**	**−145.0 (−750.0; 15.2)**	**21.0**	**< 0.001**
Basophils (cells/μl), median (IQR)	**10 (10; 23)**	**30 (20; 40)**	**−37.5 (−145.8; 0.0)**	**28.0**	**< 0.001**
Lymphocytes (cells/μl), median (IQR)	**900 (630; 1353)**	**1435 (1075; 1858)**	**−42.3 (−120.4;−12.5)**	**10.2**	**< 0.001**
CRP (mg/l), median (IQR)	**60.9 (28.4; 116.9)**	**21.4 (9.0; 39.2)**	**72.7 (24.0; 85.8)**	**42.2**	**< 0.001**
IL-6 (pg/ml), median (IQR)	**29.4 (11.4; 53.2)**	**18.2 (9.4; 34.0)**	**37.7 (−41.3; 79.5)**	–	**0.009**
BNP (pg/ml), median (IQR)	**1333.5 (539.6; 2644.4)**	**713.0 (306.0; 1277.6)**	**48.5 (22.3; 64.0)**	**10.0**	**< 0.001**
TnI (ng/ml), median (IQR)	**0.095 (0.039; 0.203)**	**0.038 (0.022; 0.083)**	**58.3 (21.4; 86.8)**	**14.1**	**< 0.001**
Hemoglobin (g/dl), mean (SD)	11.6 (1.9)	11.8 (1.6)	−2.4 (9.9)	2.9	0.282
Hematocrit (%), mean (SD)	35.9 (5.8)	36.3 (4.3)	−2.2 (10.1)	2.7	0.416
MCHC (g/dl), mean (SD)	32.5 (1.3)	32.6 (1.4)	−0.3 (3.0)	1.1	0.516

IQR, interquartile range; SD, standard deviation; C1i, C1 inhibitor; CRP, C-reactive protein; IL, interleukin; BNP, B-type natriuretic peptide; TnI, Troponin I; MCHC, Mean corpuscular hemoglobin concentration.
Variation represents the median difference value between the admission and discharge values.

but with lower variation than the biological variation (as in the group of all patients—Table 2).

P values resulting from comparison at both moments for both subgroups and for all patients between both moments are presented for comparison in Table 5. Laboratory values variation between both moments of evaluation for the group of all patients as well as the accepted biological variation is also presented. From all the markers in the study, only the complement proteins C3 and C4, eosinophils, and BNP had no significant differences between the subgroups in the study (differences independent of the presence of infection, both *P* values higher than 0.05) and presented a higher variation than the accepted biological variation. These results are highlighted in bold.

In all groups, the mean corpuscular hemoglobin concentration (MCHC) was also evaluated, which

TABLE 4. Laboratory Values at Admission and at Discharge in Patients Without Infection and Comparison Between Admission and Discharge

Noninfected (n = 78)	Admission	Discharge	Variation (%)	Biological variation (%)	P value
C1i (mg/dl), median (IQR)	**31.0 (27.6; 34.1)**	**32.3 (29.4; 35.6)**	**−3.0 (−10.6; 4.9)**	**–**	**0.018**
C3 (mg/dl), mean (SD)	**117.0 (24.4)**	**129.8 (34.8)**	**−12.8 (28.3)**	**5.2**	**< 0.001**
C4 (mg/dl), median (IQR)	**24.4 (19.5; 30.1)**	**28.3 (22.8; 34.5)**	**−16.0 (−44.2; 1.2)**	**8.9**	**< 0.001**
Leukocytes (cells/µl), median (IQR)	7230 (6170; 8918)	6750 (5650; 8115)	4.1 (−9.8; 22.3)	11.4	0.094
Neutrophils (cells/µl), median (IQR)	5080 (3830; 7030)	4220 (3520; 5340)	11.5 (−7.8; 34.9)	17.1	0.001
Monocytes (cells/µl), median (IQR)	510 (358; 650)	530 (420; 705)	−5.9 (−44.4; 14.6)	17.8	0.049
Eosinophils (cells/µl), median (IQR)	**70 (23; 150)**	**140 (80; 230)**	**−52.8 (−136.5; 0.0)**	**21.0**	**< 0.001**
Basophils (cells/µl), median (IQR)	20 (10; 20)	20 (10; 30)	0.0 (−100.0; 0)	28.0	0.001
Lymphocytes (cells/µl), median (IQR)	**1175 (795; 1648)**	**1415 (1085; 1980)**	**−13.7 (−47.4; 5.7)**	**10.2**	**< 0.001**
CRP (mg/l), median (IQR)	12.0 (6.6; 20.5)	10.6 (4.0; 20.7)	28.6 (−39.8; 56.1)	42.2	0.042
IL-6 (pg/ml), median (IQR)	14.4 (9.3; 23.1)	15.6 (7.7; 14.5)	8.5 (−84.5; 50.5)	–	0.768
BNP (pg/ml), median (IQR)	**1265.1 (627.0; 2442.3)**	**536.4 (260.2; 984.0)**	**46.9 (13.8; 74.5)**	**10.0**	**< 0.001**
TnI (ng/ml), median (IQR)	**0.029 (0.014; 0.070)**	**0.021 (0.012; 0.035)**	**30.8 (10.4; 63.4)**	**14.1**	**0.001**
Hemoglobin (g/dl), mean (SD)	12.5 (2.2)	12.5 (2.2)	−0.9 (8.7)	2.9	0.659
Hematocrit (%), mean (SD)	38.2 (6.4)	38.1 (6.4)	−0.4 (8.9)	2.7	0.947
MCHC (g/dl), mean (SD)	32.7 (1.4)	32.9 (1.4)	−0.6 (3.3)	1.1	0.124

IQR, interquartile range; SD, standard deviation; C1i, C1 inhibitor; CRP, C-reactive protein; IL, interleukin; BNP, B-type natriuretic peptide; TnI, Troponin I; MCHC, mean corpuscular hemoglobin concentration.
Variation represents the median difference value between the admission and discharge values.

TABLE 5. Resume of Data (P values) from Comparisons Between the Group With Infection and the Group Without Infection and All Patients at Both Moment of Evaluation

	Admission (P value)	Discharge (P value)	All patients (n = 133) (P value)	All patients (n = 133) Variation (%)	Biological variation (%)
C1i (mg/dl), median (IQR)	0.036	< 0.001	<0.001	−5.3	–
C3 (mg/dl), mean (SD)	**0.619**	**0.141**	**< 0.001**	**−20.6**	**5.2**
C4 (mg/dl), median (IQR)	**0.406**	**0.742**	**< 0.001**	**−14.3**	**8.9**
Leukocytes (cells/µl), median (IQR)	0.016	0.122	0.001	7.0	11.4
Neutrophils (cells/µl), median (IQR)	0.001	0.041	<0.001	19.5	17.1
Monocytes (cells/µl), median (IQR)	0.224	0.341	0.089	−5.9	17.8
Eosinophils (cells/µl), median (IQR)	**0.081**	**0.715**	**< 0.001**	**−64.6**	**21.0**
Basophils (cells/µl), median (IQR)	0.459	0.140	<0.001	−14.6	28.0
Lymphocytes (cells/µl), median (IQR)	0.018	0.841	<0.001	−20.1	10.2
CRP (mg/l), median (IQR)	0.001	< 0.001	<0.001	38.1	42.2
IL-6 (pg/ml), median (IQR)	0.003	0.295	0.037	24.3	–
BNP (pg/ml), median (IQR)	**0.727**	**0.473**	**< 0.001**	**48.2**	**10.0**
TnI (ng/ml), median (IQR)	**< 0.001**	**0.001**	**< 0.001**	**37.8**	**14.1**
Hemoglobin (g/dl), mean (SD)	0.027	0.039	0.297	−1.5	2.9
Hematocrit (%), mean (SD)	0.038	0.063	0.631	−1.1	2.7
MCHC (g/dl), mean (SD)	0.403	0.222	0.104	−0.5	1.1

IQR, interquartile range; SD, standard deviation; C1i, C1 inhibitor; CRP, C-reactive protein; IL, interleukin; BNP, B-type natriuretic peptide; TnI, Troponin I; MCHC, mean corpuscular hemoglobin concentration.
Variation represents the median difference value between the admission and discharge values for all patients group. Bold values represent the markers with no significant differences between the subgroups in the study and with significant higher variation than the accepted biological variation.

shows no significant differences in any of the groups in the study.

DISCUSSION

Our data show, among a panel of biomarkers, that C3 and C4 complement protein levels and eosinophils counts all increase during hospitalization due to acute HF, independently of infection and biological variation. However, BNP decreased independently of infection and biological variation.

The presence of a low-grade inflammatory state in HF has been largely reported (17, 18). Although the mechanisms of its activation in HF are still unclear,

evidence suggests that myocardial damage may be chronically sustained, implying a role for inflammatory mediators (3, 19). The compensatory remodeling of HF due to pressure overload or following cardiac injury also attributes a predominant role to innate immunity in HF and in its progression (20). Our data corroborate these assumptions. The levels of the complement proteins C3 and C4, an important participant in the innate immunity response, showed a significant increase between admission and discharge. Low levels of these proteins are usually an indicator of high tissue complement turnover that can mirror active remodeling. The increase in their levels, on discharge, can indicate the resolution of the cause of hospitalization and their dynamic role in this condition.

For cellular counts, the changes that were found between the admission and discharge were not always higher than the accepted biological variation. Although the increase in leukocytes is a marker of systemic inflammation, the association with the risk of HF is limited and sparse (21). The existent evidence suggests an association between these cellular counts and risk factors related to the development of HF, such as coronary heart disease, MI, and stroke, with worse prognosis in subjects who smoke and have hypertension (21, 22). High leukocytes counts in middle-aged men were also associated with increased long-term incidence of HF hospitalizations (22, 23). Our patients only showed higher leukocytes counts at the time of admission in comparison with the discharge in the subgroup of patients with infection. This fact suggests that higher counts of leukocytes can be related with increased hospitalization of HF patients, but only on those with an infection as precipitating factor of HF. The leukocytes counts include several cellular subsets, such as neutrophils, monocytes, eosinophils, and basophils. These are known to have an important role in inflammation, being representative of the cellular innate immunity. Nevertheless, in HF, only some of them were studied and associated with this condition. An increase in neutrophils has been associated with increased incidence of acute decompensated HF in patients admitted with acute MI (24). A reduced circulating eosinophil counts is associated with poor prognosis after HF hospitalization with higher rates of all-cause mortality in these patients (25). In fact, our patients revealed these same tendencies, with significantly higher counts of neutrophils at time of hospitalization in both subgroups of patients, with or without infection. However, in the subgroup of patients without infection, the variation was lower than the accepted biological variation. Significantly lower counts of eosinophils on admission, a situation reverted upon discharge, were also found in all of the groups studied. These variations were also higher than the accepted biological variation. Unfortunately, no data are available to compare with our data. The significance of this observation probably merits further investigation.

Regarding monocytes, limited data on the matter show conflicting results. In patients with an existing diagnosis of HF, it was reported that increased monocyte counts were associated with HF and predicted worse outcomes (26, 27). However, the ARIC study found no association between monocytes and incident HF (23). In our data, monocyte counts only showed a weak significant difference during hospitalization in the subgroup of patients without infection. This may represent the well-known state of systemic inflammation in HF patients and the well-known role of these cells in the immune response. However, it is now widely known that monocytes are a heterogeneous population of cells, each with distinct phenotypes and functions (28). This may be important in understanding their specific role in HF. Recent attention has been particularly directed toward the "intermediate" (CD14++CD16+CCR2+) monocytes, which appear to have prognostic significance in cardiovascular disease (29). Although in our data, monocytes counts did not revealed any statistical significant changes, it does not mean that their subpopulations did not changed. Thus, a possible and important role of monocytes in HF should not be discarded.

The presence of the inflammatory process is also clear in the subgroup of patients with infection, with higher levels of the proteins CRP and IL-6 at admission than at discharge. In patients without infection, CRP did not reveal a significant decrease between admission and discharge, independent of biological variation. However, the association between CRP and HF has already been reported, with high levels of CRP as an indicative of severity of disease (11). CRP mediates several protective processes, but may also have deleterious effects in HF, such as the upregulation of TNF-α and IL-6, which are a strong sign of this activation (3, 19, 30). In fact, circulating levels of proinflammatory cytokines are enhanced in the failing myocardium, in both ischemic and nonischemic HF, and are long known to be related to disease severity and to predict poor survival (31, 32).

Also BNP, produced by the heart in the ventricles and released in HF congestion due to cardiac chamber stretch, and TnI, a specific marker of myocardial injury, as expected (33), showed a significant difference between admission and discharge, being higher on admission in both subgroups and independent of the presence of infection.

Hemodilution can also be a major confounder factor in our study, once it is expected that excess water had been removed during hospitalization. To exclude this confounder, we evaluated MCHC in all patients and compared it between admission and discharge and between groups, and no significant differences were observed. Hence, this suggests that there was no hemodilution effect in this study.

Few registries and clinical trials have prospectively monitored parameters of innate immunity during hospitalization. To our knowledge, this study is the first to evaluate together both the humoral and cellular parts of innate immunity upon admission and at discharge from hospitalization in a population suffering from acute HF. However, we acknowledge several limitations. The small number of patients clearly represents an important limitation. The recruitment of patients and collection of samples both on admission and at discharge are a quite difficult task. It depends on various factors, such as the time of hospitalization of the patient, the outcome, the correct and in time delivered informed consent, and availability of the on-call physician. The recruitment of patients from a single center can also reveal a limitation. Although our data lack the potential to be external validated, we are strongly convicted that it is an essential starting point for larger, multicenter studies to be appropriately planned and powered. Furthermore, our results are in agreement with our previous study, where we document the prognostic value of C3 and C4, at discharge, as predictors of death patients suffering from an acute episode of HF (14). Therefore, it is consistent with the hypothesis that these proteins are involved in the progression of HF.

Our results suggest that upon admission due to an episode of acute HF, C3, C4, and eosinophils have lower levels than at discharge from hospitalization, independent of infection or biological variation. These data help to support and reinforce that these innate immunity effectors are active participants in HF and may have an important role in the resolution of the acute state of HF.

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